Amendment dated October 28, 2009 Reply to Office Action of July 28, 2009

AMENDMENTS TO THE SPECIFICATION

Please replace the abstract on page 106 with the amended abstract attached hereto as a separate sheet pursuant to 37 CFR § 1.72.

In the specification at page 1, line 1, please delete the title and replace it with the following amended title:

IMPROVED CONSTRUCTS FOR MARKER EXCISION BASED ON METHOD FOR PRODUCING MARKER-FREE TRANSGENIC PLANTS BY USING DUAL-FUNCTIONAL SELECTION MARKER D-AMINO ACID OXIDASE.

In the specification at page 92, line 41, please replace the paragraph starting with "Soluble proteins" with the following amended paragraph:

Soluble proteins were extracted by shaking 0.1 g samples of plant material that had been finely pulverized in a 1.5 ml Eppendorf EPPENDORF tube in 1 ml of 0.1 M potassium phosphate buffer, pH 8. DAAO activity was then assayed as follows. Reaction mixtures were prepared containing 2,120 µl of 0.1 M potassium phosphate buffer, pH 8, 80 µl of crude protein extract and 100 µl of 0.3 M D-alanine. The samples were incubated for 2 h at 30 °C. The enzyme activity was then assessed, by measuring the increase in absorbance at 220 nm (E = 1.090 M⁻¹ cm⁻¹) associated with the conversion of D-alanine to pyruvate, after transferring the test tubes to boiling water for 10 min to stop the reaction. In control reactions, D-alanine was added immediately before boiling. One unit of DAAO activity is defined as the turnover of one micromole of substrate per minute, and activity was expressed per gram plant biomass (fresh weight). The breakdown of D-isoleucine and D-valine in DAAO incubations, and the associated production of 3-methyl-2-oxopentanoate and 3-methyl-2-oxobutanoate, were analyzed by high-performance liquid chromatography. In other respects the reactions were carried out as described above.